Remarks/Argument

The time period for responding to the March 5, 2004 Office Action has been extended by two months to November 5, 2004 by virtue of the Notice of Appeal that was filed in the instant case on September 3, 2004. In lieu of filing an Appeal Brief, Applicants are filing the present response along with a Request for Continued Examination under 37 C.F.R. 1.114. The Applicant intends that this reponse supercede any previously submitted, but un-entered, reponses. Therefore, please ***do not*** enter any previously submitted, but un-entered, reponses.

Claims 45-77 and 96-100 are pending in the application. Claims 45, 50, 51, 59, 60, 75, 97, 98, 99 and 100 have been amended. Support for amended claim 45 is found on pg. 2, lns. 13-14; pg. 8, lns. 10-11; pg. 11, ln. 15 and pg. 17, lns. 14-16 of the specification. Support for amended claims 59 and 60 is found on pg. 14, ln. 9 of the specification. Support for amended claim 97 is found on pg. 14, lns. 13-14 of the specification. No new matter has been added by these amendments.

Claims 55-58, 61-64 and 96 have been canceled without prejudice.

New claims 101-106 are presented. Support for new claim 101 is found on pg. 2, lns. 14-15; pg. 8, lns. 12-15, and pg. 14, ln. 8. Support for new claims 102 - 105 is found on pg. 11, ln. 17 to pg. 12, ln. 4 and on pg. 12, ln. 14 to pg. 13, ln. 2 of the specification, respectively. Support for new claim 106 is found on pg. 29, ln. 20 to pg. 30, ln. 6 of the present specification. No new matter has been added.

Upon entry of this amendment, claims 45-54, 59, 60, 65-77 and 97-106 will be pending. Based on the above changes and the remarks presented below, the Applicant respectfully requests reconsideration of the claims.

The Applicant's representative thanks Examiner Kam for discussing this case by telephone, for the detailed Advisory Action mailed on September 3, 2004, and for conducting the interview on October 4, 2004. Examiner Kam indicated that she would consider new claims which recite specific embodiments of the present medicaments (that is, new claims 101-103), and a Declaration by Applicant, Leonard Girsh, M.D., which shows treatment of a patient with a medicament of the invention. The Declaration of Dr. Leonard Girsh is submitted herewith as Exh. D.

Response to section 112, 2nd paragraph rejections

Claims 45-77 and 96-100 are rejected under 35 USC 112, 2nd paragraph as allegedly being indefinite for reciting the phrase "when healthy, by a characteristic amino acid molar ratio for the healthy tissue per se, or for at least one peptide, polypeptide or protein thereof." Claims 55-58, 61-64 and 96 have been canceled, and the rejection will therefore be discussed with respect to claims 45-54, 59, 60, 65-77 and 97-100. Specifically, the Examiner noted that the rejected claims did not identify the tissue or protein from which the recited amino acid ratio was derived.

Claim 45 has been amended to recite that the ratio of the plurality of L-amino acids in the claimed medicament is characteristic of human breast milk proteins. As discussed below in the "Response to section 112, 1st paragraph enablement rejection," the amino acid ratio of breast milk proteins is known in the art. Claim 45 now identifies both the tissue and protein in the tissue from which the claimed amino acid ratio is derived, and one skilled in the art would be aware of the claimed amino acid ratio, Claim 45 (and its dependent claims 46-54, 59, 60, 65-77 and 97-100) are therefore clear and definite. Applicants respectfully request that the 35 USC 112, 2nd paragraph of these claims be withdrawn.

New claims 101-103 have been added, which specify L-amino acid ratios for the medicinal compound cyclosporin, for skin, and for the blood protein fibrinogen, respectively. As the amino acid ratios in claims 101-103 are explicitly stated, these claims are also clear and definite.

Claims 56, 57 and 61-64 are rejected under 35 USC 112, 2nd paragraph as allegedly indefinite for reciting that the claimed aliphatic side chain is a fatty acid. Claims 56, 57 and 61-64 have been canceled, and thus the rejection is moot.

Claim 97 is rejected under 35 USC 112, 2nd paragraph as allegedly being indefinite for reciting "L-gamma aminobutyric acid." This claim has been amended to read "gamma aminobutyric acid," and the Applicant respectfully requests that the indefiniteness rejection of this claim be withdrawn. Claim 99 has also been amended to change "L-gamma aminobutyric acid." to "gamma aminobutyric acid" and "L-betaine" to "betaine."

Claims 98 and 100 are rejected under 35 USC 112, 2nd paragraph as allegedly being indefinite for reciting "or and." The word "or" has been deleted from these claims in this context, to indicate that the claim recites a Markush group. The Applicant respectfully requests that the 35 USC 112, 2nd paragraph rejection of claims 98 and 100 be withdrawn.

Response to section 112, 1st paragraph enablement rejection

Claims 45-77 and 96-100 are rejected under 35 USC 112, 1st paragraph as allegedly being non-enabled for an anabolic medicament for treating damaged tissue, comprising at least one mucopolysaccharide compound in an amount which is effective to act as an anti-neo-inflammatory and anti-neo-angiogenetic agent; at least one polar surface active lipid; and a plurality of amino acids, no more than 10% of which are in the D-form, in a molar ratio which is characteristic in healthy tissue of the type being treated for damage. The Applicant respectfully traverses the rejection. Claims 55-58, 61-64 and 96 have been canceled, and the rejection will therefore be discussed with respect to claims 45-54, 59, 60, 65-77 and 97-100.

According to the Examiner, the present specification provides no guidance regarding the molar ratio of amino acids in the claimed compositions for treatment of damage to a given tissue. The Examiner also contends that the claims encompass "unspecified variants regarding the identities and amounts of components in the anabolic composition," and that the effects of the claimed compositions on damaged tissue are not adequately described. Finally, the Examiner contends that "the specification has not shown the effect of the (claimed) anabolic composition, especially in that no working examples have been provided, and that the effect of the claimed composition is "highly unpredictable."

Claim 45 has been amended to recite that the ratio of the plurality of L-amino acids in the claimed medicament is characteristic of human breast milk proteins.

The ratio of L-amino acids in human breast milk proteins was known to one of skill in the art as of the filing date of the present application. For example, the infant nutritional supplement Neocate® (available from SHS North America, Gaithersburg, MD) contains proportions of L-amino

acids based on human breast milk. See, *e.g.*, page 2 of the printout of Bines et al., 1998, *J. Pediatr. Gastroenterol. and Nutr.* 26(2): 123-128 (Exhibit A), and Table 2 therein. As the tables of Bines et al. are difficult to read in the attached printout, the relevant portion of Bines et al. Table 2, showing the L-amino acid proportions of Neocate® (which mimic that of breast tissue), are provided in Exhibit B.

The amount of at least one mucopolysaccharide compound which is effective to act as an anti-neo-inflammatory and anti-neo-angiogenetic agent, and the at least one polar surface active lipid (which is also believed to have anti-inflammatory activity) can also be readily ascertained by one skilled in the art by monitoring the degree of inflammation in and around the tissue being treated. Techniques for monitoring tissue inflammation are well-known in the art. Exemplary amounts of the at least one mucopolysaccharide compound and the at least one polar surface active lipid for use in the claimed medicaments are given on pgs. 24-25 and in the working examples. Thus, one skilled in the art can identify the appropriate amounts of the at least one mucopolysaccharide compound and the at least one polar surface active lipid for use in the claimed medicaments by merely routine experimentation.

One skilled in the art would also understand that the presently claimed medicaments can be used to treat most types of damaged tissue. As stated in the present application, at pg. 13, lns. 5-8 and at pg. 13, ln. 17 to pg. 14, ln. 1, respectively (emphasis added):

> [I]t is believed that the inventive therapeutic formulations work to promote tissue repair by providing ***stem cells*** with the optimal ratios and proper stereoisomer form of amino acids that are needed to synthesize new tissue . . .
>
> [B]y altering the balance of free L amino acids such that under the law of mass action, protein synthesis is favored over proteolysis. By adding additional free amino acids, the activity of enzymes involved in protein synthesis and degradation, such as proteases, is driven in the direction of protein synthesis and therefore in the direction of tissue production rather than protein degradation. Also, it is believed that the addition of L amino acids inhibits or arrests the catabolic protein degradation reactions of these enzymes.

One skilled in the art would be aware that most adult tissues contain a population of stem cells, which can be stimulated to effect tissue repair. See, *e.g.*, Robbins Pathologic Basis of Disease (Cotran et al., eds.), WB Saunders Co., Philadelphia, pp. 33 and 91 (Exhibit C), which state, respectively, that:

> [A]dult livers contain a small population of *stem cells* located in the junction between hepatocytes and the smallest segments of the biliary tree.
>
> *Continuously dividing cells* (also called *labile cells*) follow the cell cycle from one mitosis to the next and continue to proliferate throughout life, replacing cells that are continuously being destroyed. *Tissues that contain labile cells include surface epithelia, such as stratified squamous surfaces of the skin, oral cavity, vagina, and cervix; the lining mucosa of all the excretory ducts of the glands of the body (e.g., salivary glands, pancreas, biliary tract); the columnar epithelium of the gastrointestinal tract and uterus; and the transitional epithelium of the urinary tract and cells of the bone marrow and hematopoietic tissues.* ***In most of these tissues, regeneration is derived from a population of stem cells,*** which have an unlimited capacity to proliferate and whose progeny may undergo various streams of differentiation.

See also Fig. 1 and pg. 28, ln. 23 to pg. 29, ln. 4 of the present specification, which shows that the locus of congenital biliary atresia disease is close to the location of liver stem cells, and is in approximately the area of therapeutic activity. Likewise, the present specification at pg. 29, lns. 5-9 states that the presently claimed medicaments are effective in treating bronchial asthma, as this disease exhibits inflammation or blockage of bronchioles which are located in close proximity to stem cells in the lung. Thus, as stated on pg. 29, ln. 20 to pg. 30, ln. 6 of the present specification (emphasis added):

> [T]he essential components of the therapeutic formulations ***promote favorable substrate nutrition in vivo as well as in vitro for stem cells to thrive in tissue repair, replacement and regeneration.*** Such effects are believed to occur in mesodermal and mesenchymal tissue as well as endodermal surfaces, such as the respiratory tract. Asthma may be treated. Ailments of the GI tract such as regional ileitis (Crohn's Disease), and other inflammatory bowel diseases, including ulcerative colitis, mucous colitis, and liver diseases such as, but not limited to, congenital biliary atresia, are all believed to be amenable to treatment with the inventive formulations

and therapy. The inventive therapy is particularly advantageous for inflammatory bowel diseases that are very resistant to present therapies.

Thus, one skilled in the art would be able to make and use an anabolic medicament for treating damaged tissue, as recited in the present claims. One skilled in the art would also understand that the claimed medicaments can treat virtually any mammalian tissue, specifically human tissue, as these tissues contain stem cells which can be stimulated by the claimed medicaments to repair tissue damage.

The Examiner cites to an alleged lack of working examples as showing that the present specification does not teach how to make and use the claimed medicaments. However, "the specification need not contain an example if the invention is otherwise disclosed in such a manner that one skilled in the art will be able to practice it without an undue amount of experimentation." MPEP 2164.02. As discussed above, the specification contains ample guidance to allow one skilled in the art to make and use the claimed medicaments.

In any case, the effect of the presently claimed medicaments is illustrated in the attached Declaration submitted by the Applicant, Dr. Leonard Girsh, which shows the actual treatment of a 71-year-old female patient suffering from Crohn's disease (Exh. D). The Applicant administered to this patient a medicament according to the present invention, comprising about 10.6 g Neocate infant formula containing L-amino acids and glycine, in the genetic code and molar ratio of breast milk protein from human breast tissue; about 50-100 mg lecithin; about 12.5-40 mg phosphatidyl choline; about 225 mg EPA from fish oil; 500 mg flaxseed oil (equivalent of about 275-325 mg linolenic acid); and extracellular matrix components comprising collagen, proteoglycan aggregate complex of cartilage and chondroitin sulfate (shark cartilage 740 mg per capsule, twice daily). As discussed in the Declaration, treatment with this composition caused a significant improvement in the Crohn's disease patient, and the patient was able to take a much-reduced dose of anti-inflammatory corticosteroids.

It was well-known at the time the present application was filed that a reduction in inflammation and the clearing of symptoms in Crohn's disease, such as those detailed in the declaration, are indicative of tissue healing. See paragraphs 9-10 of the Declaration. Moreover, as

discussed above, the administration of the presently claimed medicaments encourages anabolism in the patient.

Thus, as shown by the treatment of the Crohn's disease patient presented in the Declaration, the claimed medicaments promote tissue healing by reducing inflammation while a the same time stimulating anabolic processes. As discussed above, these medicaments can be readily made and used by one skilled in the art, without undue experimentation.

Claims 45-54, 59, 60, 65-77 and 97-100 are therefore enabled for an anabolic medicament for treating damaged tissue, comprising at least one mucopolysaccharide compound in an amount which is effective to act as an anti-neo-inflammatory and anti-neo-angiogenetic agent; at least one polar surface active lipid; and a plurality of amino acids, no more than 10% of which are in the D-form, in a molar ratio which is characteristic human breast milk protein. The Applicant respectfully requests that the 35 USC 112, 1st paragraph rejection of these claims be withdrawn.

Specific L-amino acid molar ratios for use in preparing the present medicaments are also disclosed in the specification. For example, a molar ratio of L-amino acids for treating damaged skin is given on pg. 11, ln. 17 to pg. 12, ln. 4 of the specification, and for treating blood clotting deficiencies is given on pg. 12, ln. 14 to pg. 13, ln. 2 of the specification. These represent the L-amino acid molar ratios of healthy skin and for the blood protein fibrinogen, respectively. New dependent claims 102 - 103 have been added which separately recite these molar ratios. As the tissue type being treated and/or the L-amino acid molar ratios are expressly recited in new claims 101 and 102, these claims are enabled.

Pending claims 59, 60, 75, 99 and 100, and new claim 101 specify that the L-amino acid ratio in the claimed medicament is that of cyclosporin. As disclosed on pg. 14, lns. 3-4, cyclosporin comprises nonpolar cyclic oligopeptides that have immunosuppressant activity. Page 14, lns. 8-9 disclose that a molar L-amino acid ratio that produces cyclosporin effects is 2 moles L-valine; 4 moles L-leucine; and 2-moles L-alanine[1]. These values are recited in new claim 101. Because a

[1] The Examiner asked Applicant's representative to confirm this amino acid molar ratio for cyclosporin. Applicant's representative has no reason to doubt the accuracy of the amino acid molar ratio for cyclosporin as disclosed and claimed in the present application.

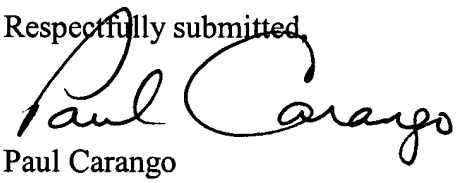
molar L-amino acid ratio is expressly recited in new claim 101, this claim and claims 59, 60, 75, 99 and 100 (which depend from it) are enabled.

New claim 104 specifies that the damaged tissue is selected from the group consisting of skin, eye, liver, gastro-intestinal, kidney, lung, and connective tissue. New claim 105 specifies that the damaged gastro-intestinal tissue is bowel tissue, and new claim 106 specifies that the bowel tissue is damaged from regional ileitis (Crohn's Disease), inflammatory bowel disease, ulcerative colitis, or mucous colitis. As discussed above, these tissues (especially the liver and gastro-intestinal tissues) are known to contain stem cells, and can therefore be treated with the presently claimed medicaments. New claims 104-106 are therefore enabled.

Conclusion

Based on the foregoing, all claims are believed to be in condition for allowance. An early action toward that end is earnestly solicited.

Respectfully submitted,



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Encls.: ***Exhibit A*** - Bines et al., 1998, *J. Pediatr. Gastroenterol. and Nutr.* 26(2): 123-128 showing L-amino acid ratio of Neocate.
Exhibit B - Excerpt from Table 2 of Bines et al., 1998, *J. Pediatr. Gastroenterol. and Nutr.* 26(2): 123-128 showing L-amino acid ratio of Neocate.
Exhibit C – Robbins Pathologic Basis of Disease (Cotran et al., eds.), WB Saunders Co., Philadelphia, pp. 33 and 91.
Exhibit D - Declaration of Leonard S. Girsh, M.D.

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Reducing Parenteral Requirement in Children with Short Bowel Syndrome: Impact of an Amino Acid-Based Complete Infant Formula

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ABSTRACT TOP

Background: The aim of this study was to assess the impact of an amino acid-based complete infant formula on enteral feeding tolerance and parenteral nutrition requirement in children with severe short bowel syndrome.

Methods: Four children (23 months-4.75 years) with short bowel syndrome who required long-term parenteral nutrition due to persistent feeding intolerance while receiving an extensively hydrolyzed formula were assessed before and after the commencement of an amino acid-based complete infant formula for a mean follow-up period of 48 months (range 39-51 months). Assessment included clinical monitoring of feeding tolerance and nutritional status, biochemistry, stool analysis, skin-prick testing to common food antigens, esophagogastroduodenoscopy and colonoscopy or jejunoscopy with biopsies, and measurement of disaccharidase levels and intestinal permeability.

Results: All patients ceased parenteral nutrition within 15 months as a result of decreased stool output and resolution of vomiting. Patients had a reduction in hospitalization (mean: 198 versus 98 days/patient/year), episodes of proven(mean: 4.3 versus 3.3/patient/year) and suspected (mean: 6.5 versus 4.0/patient/year) bacterial sepsis and central line insertions (mean: 2.5 versus 1.5/patient/year). Intestinal permeability to lactulose fell markedly(mean: 69% versus 2.7%). Disaccharidase levels increased in all three patients undergoing repeat studies.

Conclusions: An amino acid-based complete infant formula improved feeding tolerance and eliminated the need for parenteral nutrition in four children with short bowel syndrome who had previously required long-term parenteral nutrition. The clinical improvement was mirrored by improvement in measurements of intestinal function.

Article Outline

One of the important challenges in the management of infants and children with short bowel syndrome is to achieve the transition from parenteral nutrition to enteral nutrition and ultimately normal diet. Despite growing experience in providing long-term parenteral nutrition to children with short bowel syndrome, this therapy is associated with significant morbidity, mortality, and financial cost(1-8). The aim of parenteral nutrition therapy is to provide nutritional requirements for normal growth and development while the bowel undergoes adaptation necessary for the transition to an enterally based diet (1,2,9). Unfortunately, for some children this is not achieved, and they require total or partial parenteral nutrition therapy(1-4). This complex group of patients presents a significant clinical, social, and economic challenge.

Feeding recommendations for infants with severe short bowel syndrome include small volume bolus or continuous infusions of an extremely hydrolyzed formula (1,2,4,10,11). However, some children have persistent feeding intolerance on this regimen, which limits

advancement of enteral feeding. Factors that may contribute to feeding intolerance in short bowel syndrome include increased intestinal transit; impaired absorption of macromolecules, electrolytes, and water; increased osmotic load; bile salt deficiency; and gastric hypersecretion(10,12-14). Infants undergoing abdominal surgery are also reported to have a higher incidence of cow's milk protein intolerance compared with normal infants (15). Recently, IgE- and non-IgE-associated hypersensitivity have been reported in infants receiving extensively hydrolyzed formulas (16). Elemental formulas are commonly used in adults with short bowel syndrome, but until recently an elemental formula designed to meet the specific needs of infants has not been commercially available. To assess whether protein hypersensitivity to the extremely hydrolyzed formulas contributes to persistent feeding intolerance in children with short bowel syndrome, we studied the effect of an amino acid-based complete infant formula (Neocate; Scientific Hospital Supplies, Liverpool, U.K.) on the enteral feeding tolerance of a group of long-term parenteral nutrition-dependent children with short bowel syndrome.

MATERIALS AND METHODS TOP

Patients between 6 months and 5 years of age who had congenital or surgical short bowel syndrome and who had required total or supplementary parenteral nutrition for more than 6 months were eligible for this study. For inclusion into this study, patients were required to demonstrate feeding intolerance when receiving a continuous 24-hr enteral infusion of an extensively hydrolyzed formula over a minimum of a 1-mo period. Feeding intolerance was defined as (a) vomiting: more than three times per day or a volume of more than 20% of their daily enteral intake; (b) watery diarrhea more than three times per day; and/or (c) severe ulcerative buttock rash unresponsive to topical and antifungal agents, which prevented the advancement of formula volume or concentration. Informed consent was obtained from the parents of children invited to participate in this study.

Four children (three boys, one girl) ages 23 months to 4.75 years were enrolled in the study between December 1992 and March 1994(Table 1). The patients were clinically assessed as having severe short bowel syndrome based on the length of residual small intestine, and had required parenteral nutrition continuously since birth. Before the study, all patients had demonstrated persistent feeding intolerance despite multiple changes in enteral formulas and feeding protocol. These four children had spent an average of 88% of days since birth as hospital inpatients, which represented a cumulative hospital inpatient stay of 4539 days. One patient received home parenteral nutrition (patient 4). They had required an average of eight central venous line placements per patient for parenteral nutrition administration; this included one patient who had required thoracotomies for line placement because of lack of alternative line sites. All patients also received a proportion of their nutrition via the gastrointestinal tract from a continuous nasogastric (n = 1) or gastrostomy tube (n = 3) infusion of Pregestimil or 3232A (Mead Johnson, Evansville, IN, U.S.A.). The parenteral nutrition contributed 40% of their total energy and 43% of total protein plus amino acid intake. Feeding advancement was restricted by vomiting (patients 2 and 3), diarrhea (patients 1, 2, 3, and 4), and severe buttock excoriation (patient 3).

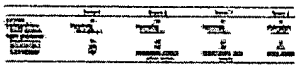


TABLE 1. *Patient profile*

The study formula was a nutritionally complete infant formula (Neocate; Scientific Hospital Supplies, Liverpool, U.K.) composed of synthetic L-amino acids with proportions based on the amino acid profile of human breast milk, and was supplemented with glutamine (Table 2). The carbohydrate was maltdextrin, and the fat component was derived from hybrid safflower oil, refined coconut oil, and oil. The vitamin and mineral profile reflected that found in breast milk. At the recommended concentration(one scoop or 5 g-30 ml of water; ≈20 kcal/30 ml), the osmolarity of the formula is 353 mOsm/kg water.

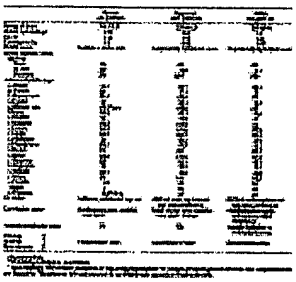


TABLE 2. *Comparison of the chemical composition of the formulae per 100 ml*

Patients had a baseline assessment before or within 8 days of commencement of the study formula. This was repeated at ≈3 mo after starting the study formula (range 2-4 mo). A full medical, surgical, and nutritional history and examination was performed. Height and weight were compared with percentiles for age and gender, and z scores were calculated using the ANTHRO Pediatric Anthropometry Software program (17,18). Blood was taken for full blood examination, liver function tests, and levels of serum albumin, calcium, magnesium, phosphate, urea, and electrolytes. Feces were obtained for microscopy, viral antigen studies, bacterial culture, and *Clostridium difficile* toxin assay. Skin prick tests to common foods(milk, egg, wheat, peanut) and inhalants were performed(16).

Intestinal permeability was assessed by measuring lactulose/rhamnose excretion. This method measures the urinary excretion of lactulose and rhamnose 5 hr after enteral administration of a solution containing 5 g of lactulose (67% wt/vol: Duphalac syrup, Duphar B.V., Weesp, The Netherlands), 1 g L-rhamnose (R-3875; Sigma, St. Louis, MO, U.S.A.) and 22.6 g of glucose brought to 100 ml with

distilled water (1,500 mOsm) as previously described(19). In the normal intestine, lactulose does not transit the paracellular tight junctions of the small intestine and is not found in significant amounts in the urine. Rhamnose is a small molecule that is transported through the normal enterocyte and appears in the urine in amounts proportional to the ingested dose. If there is distrubance of intestinal barrier function, as occurs with inflammation of the mucosa, lactulose may transit the mucosa and appear in higher quantities in the urine, whereas rhamnose absorption may be disturbed and decreased amounts may be found in the urine. Results are expressed as lactulose and rhamnose present in the urine as a percentage of the ingested dose and as the ratio of urinary lactulose to rhamnose. Patients all underwent esophagogastroduodenoscopy and colonoscopy, or jejunoscopy in the patient with a stoma. Duodenal fluid was collected to examine for pH and by microscopy. Endoscopic biopsies were obtained at standardized levels from the esophagus, gastric corpus and antrum, duodenum, and at regular intervals in the colon and/or distal small intestine. Maltase, sucrase, and lactase levels were assayed from duodenal biopsies(20).

During week 1, the patients were given a 24-hr enteral infusion of a 2% glucose solution at the same total volume and infusion rate as their usual formula in attempt to establish baseline stool output and vomiting pattern on a consistent volume of enteral fluid intake (n = 3). Patient 4 was receiving home parenteral nutrition and his parents requested that he not receive the week of 2% glucose solution administration. Parenteral nutrition was increased to provide full nutritional requirements during this period. Oral intake was initially limited to a single food type (potato) to minimize the potential effect of oral diet on feeding tolerance. All stool output and vomiting was recorded. During week 2, the study formula was introduced at the same infusion rate, initially as a dilute formula (half strength with water). As the concentration and volume of formula was advanced, parenteral nutrition was reduced. Oral diet was commenced with the weekly addition of a new food, initially vegetables and rice, for the first month. If tolerated, the diet was liberalized to include chicken, meat, and fruit. Stool output, vomiting, buttock condition, weight, and parenteral and enteral intake were monitored daily for the first month. Baseline studies were repeated once the patient had a stable stool output (less than four stools per day or <500 ml of jejunostomy output) while receiving full volume (more than or equal to prestudy enteral volume) infusion of the study formula at a concentration of ≥20 kcal/30 ml (mean 3 mo; range 2-4 mo). Studies were repeated while the patients were receiving the study formula. Anthropometry, feeding tolerance, parenteral, enteral, and oral intake, intercurrent illness, surgical interventions, and hospital admissions were recorded at regular intervals for a mean follow-up period of 48 mo (range 39-51 mo). Statistical analysis was performed using the Wilcoxon ranksum test for nonparametric data.

RESULTS TOP

All patients were able to cease parenteral nutrition within 15 mo of initiating the study formula (mean 12 mo) (Fig. 1). The discontinuation of parenteral nutrition was not at the expense of weight gain or growth, with no significant change shown in the z score for weight and height (Table 3). After a mean follow-up period of 48 mo (range 39-51 mo), only one patient (patient 3) has subsequently required temporary parenteral nutrition during two separate intercurrent infections.

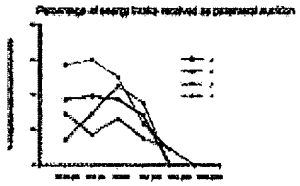


FIG. 1. Percentage of energy intake received as parenteral nutrition.

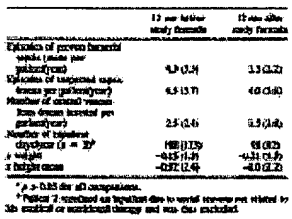


TABLE 3. *Clinical outcome: mean (standard deviation)* [a]

Stool output dramatically decreased in all patients within 1 mo of starting the study formula (Table 4). Daily jejunostomy output fell from 53 to 19 ml/kg/day in patient 1 and stool frequency reduced from a mean of 7 to 2.5 stools per day in patients 2, 3, and 4. There was complete resolution of recurrent vomiting in the two affected patients. Recurrent metabolic acidosis in patient 4 had resolved. Patient 2 had persistent blood-stained stool that continued 1 week after commencing the study formula. This resolved with advancement of the study formula and a limited course of oral prednisolone therapy at 2 mg/kg/day for 4 wk followed by a reducing dose.

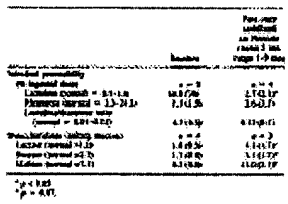
TABLE 4. *Relationship between feeding tolerance and nutritional intake*

Twelve months after commencing the study formula, all four patients still received the majority of their nutrition via enteral infusion of

the study formula; however, their oral contribution had increased from an average of 15 to 21% of energy intake. Patient morbidity and hospitalization dramatically reduced (Table 3). Serum biochemistry and stool studies showed normal results in all patients at baseline and at repeat measurement, except for mild elevations in aspartate transaminase and gamma-glutamyl transferase levels on both occasions. Of the three patients completing skin prick tests, one patient (patient 1) had a very high level of skin test reactivity consistent with anaphylactic sensitivity to cow milk and egg white.

Intestinal permeability to lactulose was markedly increased at baseline in the two patients tested. When patients (n = 4) had been stabilized on the study formula, lactulose permeability approached the normal range(Table 5). All patients had an initial esophagogastroduodenoscopy and colonoscopy or jejunoscopy, and three patients had repeat studies once they had stabilized on the study formula. The most consistent finding was a mild, nonspecific neutrophilic inflammation involving one or more areas from which biopsy specimens were taken from the upper and lower gastrointestinal tract. There were no significant changes in the type or numbers of inflammatory cells in biopsies taken from the same areas at the repeat study. Patient 3 had subtotal villous atrophy of the small intestinal mucosa. Disaccharidase levels increased in all three patients who underwent repeat endoscopy (Table 5).

TABLE 5. *Gastrointestinal studies: mean (standard deviation)*



DISCUSSION TOP

This study reports a group of four long-term parenteral nutrition-dependent children with severe short bowel syndrome who had failed in previous attempts to cease parenteral nutrition due to feeding intolerance on an extensively hydrolyzed formula. Within 15 mo of commencing the study formula, parenteral nutrition was ceased in all four patients. This was achieved as a result of decreased stool output and resolution of vomiting and severe buttock excoriation when patients received the study formula. The clinical improvement in feeding tolerance was evident within 1 mo, but parenteral nutrition was weaned slowly in these patients because of their past history of rapid clinical decompensation with increased gastrointestinal losses and of problems with intravenous access in a crisis situation. Improvement in feeding tolerance and in measurements of intestinal function within this short time frame suggests that these benefits were either an effect of the study formula or cessation of the extensively hydrolyzed formula rather than intestinal adaptation.

Within 12 mo, the patients experienced a marked reduction in hospitalization, surgical intervention, and episodes of bacterial sepsis or suspected bacterial sepsis. Furthermore, the change to a complete enteral diet was not at the expense of weight gain or growth. The reduction in morbidity has resulted in a significant social benefit to these families as well as achieving a major cost saving.

Enteral feeding in short bowel syndrome requires consideration of the length of bowel resected, the site of the resection, the presence or absence of a stoma and, as suggested in this study, consideration of the function of the residual bowel (2,10,12). Our patients had mild, nonspecific microscopic features of inflammation on endoscopic biopsy. The function of the residual bowel was also abnormal as reflected by increased lactulose permeability and reduced disaccharidase levels. Functional abnormalities of the intestine in the presence of minimal histological changes on mucosal biopsy have been reported in other diseases(21,22). In patients with a reduced length of intestine, changes in intestinal function may be associated with clinically significant problems including feeding intolerance. Once stabilized on the study formula, urinary lactulose excretion dramatically fell, and disaccharidase levels increased. These studies paralleled clinical improvement in feeding tolerance and occurred despite the absence of significant microscopic change in the degree of inflammation present on endoscopic biopsies. These findings suggest that either the formula provided something that improved gut function (such as glutamine), or did not contain a substance that resulted in a disturbance in gut function (such as a dietary protein antigen).

Abnormal intestinal permeability has been reported in adults receiving parenteral nutrition (23). The role of increased intestinal permeability in allowing bacterial translocation across the mucosa and increasing the risk of bacteremia and colonization of the central venous line has been proposed (24,25). Increased intestinal permeability has also been observed in patients with inflammatory intestinal diseases such as untreated celiac disease and Crohn's disease, infective gastroenteritis, food intolerance, and after neonatal surgery(26-28). At the initial assessment, our patients had large increases in urinary lactulose excretion, suggesting there was an increase in permeability to lactulose through intercellular tight junctions in these patients (26). In this clinical setting, dietary antigens that would normally be restricted to the intestinal lumen may be allowed potentially to pass through these leaky intercellular junctions (26). If a dietary antigen is important in perpetuating increased intestinal permeability, it is conceivable that elimination of the offending antigen may result in the resolution of this functional abnormality, leading to clinical improvement similar to that observed in our patients. Late-onset non-IgE-mediated hypersensitivity reactions to extensively hydrolyzed whey and casein formulas have been reported, and an amino acid-based complete infant formula has been found to be an effective treatment (16).

This group of four long-term parenteral nutrition-dependent children with severe short bowel syndrome successfully discontinued parenteral nutrition and experienced a reduction in morbidity when stabilized on the study formula. We have attributed this improvement to the exclusion of the extensively hydrolyzed formula. To confirm this hypothesis, double-blind, placebo-controlled studies are required, but none of the families involved in this study would agree to participate. We now routinely initiate an amino acid-

based complete infant formula in infants with short bowel syndrome who develop feeding intolerance on breast milk.

Acknowledgment: The authors thank Mr. Alex Auldist, Mr. Keith Stoker, Mr. Hock Tan, Professor John Hutson, and Dr. Peter Loughnan, who contributed their patients to this study.

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Keywords:
Infant formula; Intestinal permeability; Parenteral nutrition; Short bowel syndrome

Citing Articles TOP

Irreversible Intestinal Failure.
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Ksiazyk, Janusz *; Piena, Marjolein +; Kierkus, Jaroslaw *; Lyszkowska, Malgorzata *
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Walker-Smith, John *[P]; Barnard, John +; Bhutta, Zulfiqar *; Heubi, James +; Reeves, Ziomara ++; Schmitz, Jacques [P]
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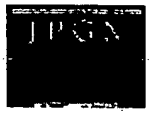

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Candy, David C. A. *; Densham, Lucy *; Lamont, Lilias S. *; Greig, Marjorie +; Lewis, Jonathan +; Bennett, Heidi ++; Griffiths, Mervyn [S]
[Fulltext] [PDF (72 K)]

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[Abstract] [Fulltext]

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[Fulltext]

Exhibit B – Excerpt from Table 2 of Bines et al., 1998, *J. Pediatr. Gastroenterol. and Nutr.* 26(2): 123-128 showing L-amino acid ratio of Neocate.

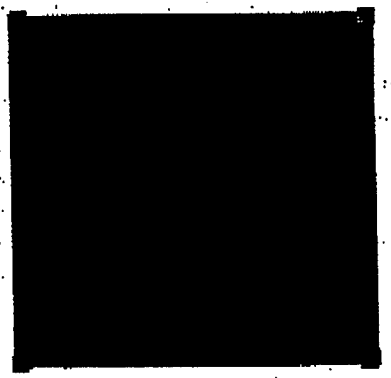
Neocate (15g/100ml)	
Protein Source	Synthetic L-Amino Acids
Protein Molecular weight (daltons)	
Mean	150
% <500	100
Maximum	250
Amino acid profile (mg)	
L-Alanine	91.5
L-Arginine	162
L-Aspartic acid	151.5
L-Cystine	60
L-Glutamic acid	184.5[b][nil]
Glycine	142.5
L-Histidine	93
L-Isoleucine	142.5
L-Leucine	244.5
L-Lysine	166.5
L-Methionine	39
L-Phenylalanine	109.5
L-Proline	174
L-Serine	106.5
L-Threonine	120
L-Tryptophan	48
L-Tyrosine	109.5
L-Valine	156
L-Carnitine	1.5
Taurine	3
L-Glutamine	16.5[b][201]

b Since November 1995 a revised formulation of Neocate has been released. The amounts in brackets indicate the amino acid composition in the new formulation. The patients in this study received the pre-1995 formula composition listed in this table.

Robbins

PATHOLOGIC BASIS of DISEASE

Sixth Edition



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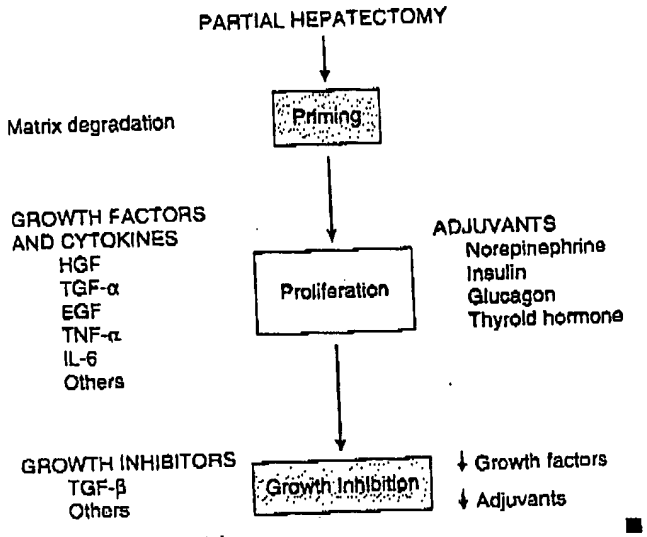

Figure 2–2

Postulated sequence of events in the compensatory hyperplasia after partial hepatectomy.

Subsequently, DNA synthesis declines, and by the time the liver mass is restored (at 1 to 2 weeks), the liver cells become quiescent again.

There is substantial evidence that cell proliferation in this setting is dependent on the action of polypeptide growth factors and cytokines (see Chapter 4 for a detailed discussion of cell growth). Factors implicated include the following:

- *Growth factors*: *Hepatocyte growth factor* (HGF) and its receptor c-Met are key factors for liver growth and function.[4] HGF (also called *scatter factor*) initially identified in the serum of hepatectomized rats as a potent mitogen for cultured hepatocytes, is produced in the liver by nonparenchymal cells and by mesenchymal cells in many organs. Plasma HGF levels rise rapidly after partial hepatectomy. Both *TGF-α* and *epidermal growth factor (EGF)* are also mitogenic for hepatocytes in culture, and experimental studies suggest that EGF may play a mitogenic role in the early stages after partial hepatectomy, while *TGF-α* acts at later times.
- *Cytokines*: *Interleukin-6 (IL-6)* and *tumor necrosis factor-α (TNF-α)* are important components of the early signaling pathways involved in regeneration. The functional relevance of IL-6 was demonstrated in transgenic mice lacking the cytokine. In these animals, defective hepatocyte regeneration results in massive hepatic necrosis after partial hepatectomy, which can be prevented by administration of IL-6.[5]

None of these growth factors or cytokines, however, are sufficient to induce proliferation in normal liver cells in vivo, and it has been proposed that an initial *priming* signal to the remnant hepatic cells is necessary for the full effect of these mitogens (Fig. 2–2). Such priming signals include degradation of the extracellular matrix, which would convert inactive matrix-associated HGF to its active, receptor binding form. Certain hormones, such as norepinephrine, whose blood level also increases after hepatectomy, and insulin, may function as adjuvants for cell proliferation. Cessation of cell growth, after the liver mass has been restored, appears to be caused by *growth inhibitors* produced in the liver itself. One of these inhibitors is TGF-β, which is produced by nonparenchymal cells of the liver.

In addition to proliferating differentiated hepatocytes, adult livers contain a small population of *stem cells* located in the junction between hepatocytes and the smallest segments of the biliary tree.[6] These stem cells have a variety of developmental options, including differentiating into hepatocytes and biliary duct epithelium. Stem cells do not play a major role in the hyperplasia after hepatectomy but may participate in the regeneration that occurs after certain forms of liver injury, such as hepatitis.

PATHOLOGIC HYPERPLASIA

Most forms of *pathologic hyperplasia are instances of excessive hormonal stimulation or are the effects of growth factors on target cells*. An example of hormonally induced hyperplasia is hyperplasia of the endometrium. After a normal menstrual period, there is a rapid burst of proliferative activity. As is well known, this proliferation is potentiated by pituitary hormones and ovarian estrogen. It is brought to a halt by the rising levels of progesterone, usually about 10 to 14 days before the anticipated menstrual period. In some instances, however, the balance between estrogen and progesterone is disturbed. This results in absolute or relative increases in the amount of estrogen, or both, with consequent hyperplasia of the endometrial glands. Although this form of hyperplasia is a common cause of abnormal menstrual bleeding, the hyperplastic process remains controlled nonetheless: If the estrogenic stimulation abates, the hyperplasia disappears. Thus, it responds to regular growth control of cells. As is discussed in Chapter 8, Neoplasia, it is this response to normal regulatory control mechanisms that differentiates benign pathologic hyperplasias from cancer. *Pathologic hyperplasia, however, constitutes a fertile soil in which cancerous proliferation may eventually arise.* Thus, patients with hyperplasia of the endometrium are at increased risk for developing endometrial cancer (Chapter 24).

Hyperplasia is also an important response of connective tissue cells in wound healing, in which proliferating fibroblasts and blood vessels aid in repair (Chapter 4). Under these circumstances, growth factors are responsible for the hyperplasia. Stimulation by growth factors is also involved in the hyperplasia that is associated with certain *viral infections*, such as papillomaviruses, causing skin warts and a number of mucosal lesions composed of masses of hyperplastic epithelium.

Hypertrophy

Hypertrophy refers to an increase in the size of cells and, with such change, an increase in the size of the

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit	: 1653	**Customer No. 035811**
Examiner	: Chih Min Kam	
Serial No.	: 09/639,859	Docket No.: IPI-04-1174R
Filed	: August 16, 2000	
Inventor(s)	: Leonard S. Girsh	
Title	: THERAPEUTIC STEM CELL GROWTH	
	: FACTOR COMPOSITION, ANTI-	
	: INFLAMMATORY COMPOSITION	
	: AND USES THEREOF	

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Leonard S. Girsh, M.D., hereby declare as follows:

1. I am the sole inventor of the above-referenced application.

2. I have read and understood the Official Action dated March 5, 2004, in which the Examiner has rejected the pending claims as allegedly lacking enablement.

3. The pending claims are directed to an anabolic medicament for treating damaged tissue. The claimed medicament comprises three components:

(a) at least one mucopolysaccharide compound in an amount which is effective to act as an anti-neo-inflammatory and anti-neo-angiogenetic agent;

(b) at least one polar surface active lipid; and

(c) a plurality of amino acids, no more than 10% of which are in the D-form, in a molar ratio which is characteristic of human breast milk protein.

4. The Examiner states in the March 5, 2004 Official Action that the present specification does not demonstrate an anabolic composition comprising the three components listed in paragraph 3 above, nor has the present specification shown the effect of such

compositions on damaged tissues. The Examiner indicated in the Official Action that, in the (alleged) absence of a demonstration in the present specification showing the use and effect of the claimed compositions, that I would have to provide experimental evidence that the pending claims are enabled.

5. I have treated a 71-year-old female patient who had suffered from Crohn's disease for more than 3 decades. This patient's symptoms included diarrhea, constipation, severe bouts of abdominal pain and fever, G.I. bleeding, generalized aching, extreme fatigue, nausea, and food and dairy intolerance, and she was being treated with 4 mg of corticosteroid, once daily, with no response. Corticosteroid dosing was then increased to 4 times daily for acute flare ups.

6. I administered to this Crohn's disease patient a medicament according to the present invention, comprising about 10.6 g Neocate infant formula containing L-amino acids and glycine, in the genetic code and molar ratio of human breast milk protein; about 50-100 mg lecithin; about 12.5-40 mg phosphatidyl choline; about 225 mg EPA from fish oil; 500 mg flaxseed oil (equivalent of about 275-325 mg linolenic acid); and extracellular matrix components comprising collagen, proteoglycan aggregate complex of cartilage and chondroitin sulfate (shark cartilage 740 mg per capsule, twice daily).

7. For ease of comparison to the elements appearing in claim 1 of the present application, I list below components of the medicament used to treat this Crohn's disease patient in terms of the language used in claim 1.

Medicament for treatment of Crohn's disease patient	**Claim 1**
extracellular matrix components comprising collagen, proteoglycan aggregate complex of cartilage and chondroitin sulfate (shark cartilage 740 mg per capsule, twice daily)	at least one mucopolysaccharide compound in an amount which is effective to act as an anti-neo-inflammatory and anti-neo-angiogenetic agent
about 50-100 mg lecithin; about 12.5-40 mg phosphatidyl choline; about 225 mg EPA from fish oil; and 500 mg flaxseed oil (equivalent of about 275-325 mg linolenic acid)	at least one polar surface active lipid
about 10.6 g twice daily of Neocate infant formula in the genetic code and molar ratio of human breast milk protein	a plurality of amino acids, no more than 10% of which are in the D-form, in a molar ratio which is characteristic of human breast tissue protein

8. Symptoms of severe abdominal pain and diarrhea, and flare-up in this Crohn's disease patient were cleared within 24 hours after treatment of the medicament described in paragraph 7. The improvement continued over the next few weeks, and the patient responded to the least amount of corticosteroids, which was alternating daily dosages of a half a tablet (2 mg) with a full tablet (4 mg) required to prevent flare-ups in the past several decades of management. Severe unsightly bruising and poor healing of lacerations and associated intolerance of sutures in the patient were also reduced.

9. It was well-known at the time the present application was filed that a reduction in inflammation and the clearing of symptoms in Crohn's disease, such as those detailed above for this patient, are indicative of tissue healing. See, for example, the attached passage from Pathology, Rubin E and Farber JL (eds.), J.B. Lipincott Co., Phila. PA, 1988, p. 68, which reads (emphasis added):

> Man is constantly subjected to injuries that may result in cell death and tissue destruction. Healing, a response to this injury, represents an attempt to maintain normal structure and function. Healing overlaps with the inflammatory process, ***and it is only for didactic purposes that the two are separated.***

10. Although components of the presently-claimed medicaments are believed to have, of themselves, anti-inflammatory activity, the tissue healing processes initiated by the claimed compositions were not solely due to a reduction in inflammation, but are also the result of anabolism. The claimed medicaments provide L-amino acids in a molar ratio which is characteristic of human breast milk proteins. As stated in the present application, at pg. 13, lns. 5-8 and at pg. 13, ln. 17 to pg. 14, ln. 1:

> [I]t is believed that the inventive therapeutic formulations work to promote tissue repair by providing stem cells with the optimal ratios and proper stereoisomer form of amino acids that are needed to synthesize new tissue . . .
>
> [B]y altering the balance of free L amino acids such that under the law of mass action, protein synthesis is favored over proteolysis. By adding additional free amino acids, the activity of enzymes involved in protein synthesis and

degradation, such as proteases, is driven in the direction of protein synthesis and therefore in the direction of tissue production rather than protein degradation. Also, it is believed that the addition of L amino acids inhibits or arrests the catabolic protein degradation reactions of these enzymes.

Thus, the claimed medicaments promote tissue healing by reducing inflammation while a the same time stimulating anabolic processes. This is in contrast to the action of anti-inflammatory drugs like aspirin or corticosteroids, which inhibit inflammation *without* stimulating anabolic processes.

11. From the clinical results and observations during the treatment of this Crohn's disease patient described above, I have therefore concluded that the anabolic processes of tissue protein synthesis and cell membrane repair/replacement had occurred as a result of administering the medicament according to the present invention described above. I have therefore successfully treated a patient with Crohn's disease with a medicament according to claim 1 of my patent application.

12. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-referenced patent application or any patent issued thereon.

10-12-04 Leonard S. Girsh M.D.

Date Leonard S. Girsh, M.D.

Pathology

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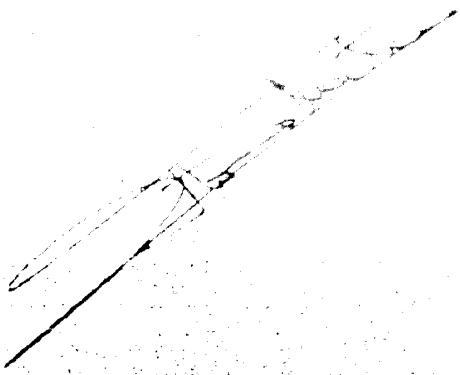
John L. Farber, M.D.

With [illegible] Contributors



Illustrations by
Dimitri Karetnikov

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Lionel and Freda Farber

and our wives:
Linda Anne and Susan

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Man is constantly subjected to injuries that may result in cell death and tissue destruction. **Healing**, a response to this injury, represents an attempt to maintain normal structure and function. Healing overlaps with the inflammatory process, and it is only for didactic purposes that the two are separated. Some primitive organisms can replace almost any cell or tissue with a new one, a process called **regeneration.** Although regeneration is, in general, a desirable process, there are potential disadvantages. For example, replacement of neurons would require cell division, an event that could result in loss of all the information gathered and stored during the lifetime of the dividing cell. If every cell could be regenerated there would be no death. On the other hand, if lost cells could not be replaced, the life span of most organisms would be drastically curtailed. Organisms exist between these two ends of the spectrum, with the balance somewhat biased toward regeneration.

When the cell membrane of an amoeba is punctured, the immediately adjacent cytoplasm condenses, sealing the defect and producing a new cell membrane. This reaction may be viewed as a primitive healing reaction. In multicellular organisms, the healing process is more complex. Invertebrates and amphibians can replace lost parts: Lobsters regrow lost claws, salamanders develop a new lens from the iris, and newts replace lost extremities. The process of regenerating whole limbs is termed **axial regeneration.**

When a newt's limb is amputated, the epidermal cells adjacent to the wound divide and rapidly cover the stump. Epithelial cell proliferation continues and the cells pile up at the apex, forming an apical cap. The connective tissue cells in the stump—fibroblasts, myocytes, and osteocytes—divide. The daughter cells lack some of the differentiated properties of the parent cells, the result of a process called **dedifferentiation.** The dense extracellular matrix originally present in the stump is catabolized and replaced by a loose, edematous stroma resembling embryonic mesenchyme. The combination of mesenchymal cells embedded in a loose, edematous stroma is termed the **blastema.** The blastemal cells multiply rapidly, endothelial cells proliferate and vascularize the blastema, and orderly differentiation into bone, muscle, tendon, arterioles, capillaries, and venules follows. The end result is the accurate replacement of the lost part.

This complex mechanism of axial regeneration is presumably controlled by the genetic information contained in the cells of the stump; amputation triggers the expression of this information, which is repressed after embryonic development. However, not all the genetic information in the cells of the stump is expressed. The cells proximal to the amputation repress the formation of proximal structures, since only the distal structures are regenerated. **As we ascend in the phylogenetic scale from reptiles to birds and mammals, repression is favored over derepression.** In mammals, granulation tissue, which replaces lost tissue, is reminiscent of the amphibian blastema. However, rather than forming a limb, granulation tissue matures only into dense connective tissue and eventuates in a scar. This replacement of lost tissue by scar tissue is termed **repair.** There are two major components of the repair reaction, the extracellular matrix and the cells.

The Extracellular Matrix

The extracellular matrix is a stable complex of macromolecules that underlies epithelia and surrounds connective tissue cells. Although the glycosaminoglycans of a bacterium's capsule constitute a primitive extracellular matrix, a complex extracellular matrix resulting from the interaction of several macromolecules is the hallmark of multicellularity. An inert glue cannot distribute and maintain cells in a predetermined and yet dynamic pattern. Only a matrix that contains information can direct migration, attachment, differentiation, and organization of the cells. The importance of the extracellular matrix for multicellularity is indicated by the production of collagen, laminin, and fibronectin as early as cleavage of the fertilized ovum. The information contained in the extracellular matrix is important not only for development but also for wound healing.

In spite of the differences in tertiary structure, physical properties, and biologic context, the matrix proteins of invertebrates, fish, reptiles, birds, and mammals share a common plan. A third of their amino acid content is glycine, and they are rich in the amino acids serine, proline, threonine, and alanine. These four amino acids are coded by RNA triplets with cytosine and uracil as the second and third residue, and differ from each other only in the first residue of the code. Proteins with great apparent disparities—the invertebrate fibroin, silk, and resilin, for example, as well as human collagens and elastin—share this common plan. Although it is conceivable that this similarity could result from evolutionary convergence, it is more likely that the present polymorphism is the result of evolution from a single primordial gene.

The extracellular matrix not only provides tissues with structural support but also exchanges information with cells, thereby modulating a host of processes, including development, cell migration, at-